and the time course of light emission was recorded. Using a luminometer it was also possible to detect the light emitted by intact bacteria in the presence of luciferin although the intensity of the light was greatly reduced in comparison to that emitted by the luciferase in cell extracts (data not shown). Presumably, this is due to a low permeability of the *E. coli* membrane to luciferin.

The smallest amount of firefly luciferase that we could detect on OG-1 X-ray film was 30 pg or 5×10^{-16} mol. This range of sensitivity could very likely be extended by using a high-speed film such as the ASA 20,000 Polaroid Land Type 612 instant film.[20] Much greater levels of sensitivity can be attained using a photomultiplier tube to detect the light emitted by the luciferase. One light unit is produced by 8.5 pg (1.4×10^{-16} mol) of luciferase when assayed in an Analytical Luminescence Laboratories Monolight 401 luminometer, and assay mixes lacking luciferase produced a background signal of 0.005 light units in the same instrument. A signal 10 times greater than the background was produced by 0.43 pg (7×10^{-18} mol) of firefly luciferase. The ease of performing assays for luciferase coupled with the great sensitivity of light detection systems make the firefly luciferase gene potentially very useful as a means of monitoring promoter activity in cells.

[20] G. H. G. Thorpe, T. P. Whitehead, R. Penn, and L. J. Kricka, *Clin. Chem.* **30**, 807 (1984).

[2] Extraction of Adenosine Triphosphate from Microbial and Somatic Cells

By PHILIP E. STANLEY

There are many reasons for extracting and measuring adenosine triphosphate (ATP) from cells, but they may be placed into one of two main categories.

1. The level of endogenous adenosine triphosphate (ATP) in a cell may be used as an index of energy status. It is therefore useful in metabolic and physiological studies.[1]

2. Estimates of cell numbers in microbial and tissue cultures may be obtained after assuming that the ATP per cell remains at a fairly constant (within a factor of five) and known value under defined conditions. Thus

[1] M. J. Harber, *in* "Clinical and Biochemical Luminescence" (L. J. Kricka and T. J. N. Carter, eds.), p. 189. Dekker, New York, 1982.

by measuring total ATP in a sample of culture, cell numbers may be rapidly obtained. This is the basis of rapid microbiology using the ATP-firefly luminescence technique.[1-5]

Extraction of ATP from cells and its subsequent measurement using the firefly luciferase procedure is often used. However there have been few studies to critically test the effectiveness of the adopted protocols for a wide range of cells.[6,7]

Properties of the Ideal Extractant

1. It should penetrate the cell wall and membrane more or less instantaneously.
2. It should extract ATP more or less instantaneously.
3. It should extract the target intracellular ATP pool completely.
4. It should instantaneously and irreversibly inactivate all enzymes that use ATP as a substrate or produce ATP from other substrates.
5. It should not cause breakdown of ATP (e.g., hydrolysis) either in short term (at the extraction time) or long term (during storage).
6. It should not have an inhibitory (quenching) effect on firefly luciferase during ATP assay.
7. It should not have an effect on the kinetics of firefly reaction.[8] Such an effect will cause problems of signal distortion and consequently internal standardization.
8. It should not extract undue quantities of extraneous materials which in themselves affect the firefly assay (by quenching or inhibition) and/or the result, e.g., colored agents, turbidity.[9]

In mixed cell populations, where, for example, bacteria and somatic cells exist, there may also be a need to selectively extract ATP from

[2] D. M. Karl, *Microbiol. Rev.* **44**, 739 (1980).
[3] A. Lundin, *in* "Clinical and Biochemical Luminescence" (L. J. Kricka and T. J. N. Carter, eds.), p. 43. Dekker, New York, 1982.
[4] H. Van de Werf and W. Verstraete, *in* "Analytical Applications of Bioluminescence and Chemiluminescence" (L. J. Kricka, P. E. Stanley, G. H. G. Thorpe, and T. P. Whitehead, eds.), p. 33. Academic Press, London, 1984.
[5] D. Slawinska and J. Slawinski, *in* "Chemi- and Bioluminescence" (J. G. Burr, ed.), p. 533. Dekker, New York, 1985.
[6] A. Lundin and A. Thore, *Appl. Microbiol.* **30**, 713 (1975).
[7] A. Lundin, *in* "Analytical Applications of Bioluminescence and Chemiluminescence" (L. J. Kricka, P. E. Stanley, G. H. G. Thorpe, and T. P. Whitehead, eds.), p. 491. Academic Press, London, 1984.
[8] L. J. Kricka and M. DeLuca, *Arch. Biochem. Biophys.* **217**, 674 (1982).
[9] W. W. Nichols, G. D. W. Curtis, and H. H. Johnston, *Anal. Biochem.* **114**, 433 (1981).

microbial or somatic cells. This imposes further constraints on the properties of the extractant.

When the sample itself is complex and perhaps variable, e.g., soil[10,11] or rumen contents,[12] this will put additional requirements on the properties of the extractant and extraction procedure.

It is worthwhile noting that properties 1 to 4 are associated with the extraction process alone whereas 5–8 have an affect on the ATP-firefly assay.

Note that properties 4 and 6 are apparently contradictory. However, the use of boiling buffer or cold acid (the latter requires immediate neutralization following extraction) does fulfill these needs but unfortunately also introduces sample dilution (and thus loss in sensitivity) or extra manipulation.

Treatment of Cells Which Affect ATP Level

Before extraction is performed it is usually necessary to take a sample; if this is not done properly ATP levels may change during this process. In addition a wide range of other factors have been shown to effect ATP levels[13] and the worker should be aware of these: (1) change of growth rate,[14] (2) change of nutient(s) or their concentration(s),[14–19] (3) change of gaseous environment, e.g., oxygen tension,[20,21] (4) change of temperature,[22] (5) change of pH,[23] (6) change of pressure,[24] and (7) change of light flux (for photosynthetic organisms).[17,25]

Laboratory techniques which one might employ to harvest or sample cells may well involve one or more of the above. I am thinking of centrifuging and filtration.

[10] J. M. Oades and D. S. Jenkinson, *Soil Biol. Biochem.* **11**, 201 (1979).
[11] J. J. Webster, G. J. Hampton, and F. R. Leach, *Soil Biol. Biochem.* **16**, 335 (1984).
[12] D. E. Nuzback, E. E. Bartley, S. M. Dennis, T. G. Nagaraja, S. J. Galitzer, and A. D. Dayton, *Appl. Environ. Microbiol.* **46** 533 (1983).
[13] P. C. T. Jones, *J. Theor. Biol.* **34**, 1 (1972).
[14] J. S. Franzen and S. B. Binkley, *J. Biol. Chem.* **236**, 515 (1961).
[15] L. Gustafsson, *Arch. Microbiol.* **120**, 15 (1979).
[16] M. Ohmori and A. Hattori, *Arch. Microbiol.* **117**, 17 (1978).
[17] A. Lewenstein and R. Bachofen, *Arch. Microbiol.* **116**, 169 (1978).
[18] C. M. M. Franco, J. E. Smith, and D. R. Berry, *J. Gen. Microbiol.* **130**, 2465 (1984).
[19] M. B. Nair and R. R. Eady, *J. Gen. Microbiol.* **130**, 3063 (1984).
[20] R. E. Strange, H. E. Wade, and F. A. Dark, *Nature (London)* **199**, 55 (1963).
[21] R. R. Mathis and O. R. Brown, *Biochem. Biophys. Acta* **440**, 723 (1976).
[22] Y. N. Lee and M. J. Colston, *J. Gen. Microbiol.* **131**, 3331 (1985).
[23] K. D. Beaman and J. D. Pollack, *J. Gen. Microbiol.* **129**, 3103 (1983).
[24] J. V. Landau, *Exp. Cell Res.* **23**, 539 (1961).
[25] P. C. T. Jones, *Cytobios* **6**, 89 (1970).

Therefore the experimental design should encompass a phase to ascertain whether or not the harvesting or sampling technique itself affects the ATP level.

Other Factors Which Affect ATP Level

These factors include the (1) age of cells or stage of growth,[23,26,27] (2) stage of cell division,[28–29] (3) density of cells,[30] (4) phage and virus[31–33] and microbial infections,[34] (5) action of agents which change cell type, e.g., tumor-promoting agents, and other agents such as (6) antibiotics[35,36] and certain drugs,[37] (7) metabolic inhibitors, cogeners of metabolites and toxins, (8) disinfectants,[38] pesticides,[39] and herbicides,[40] etc., (9) heavy metals, and (10) radiation, e.g., ultraviolet, microwave, X- and gamma rays.

General Considerations

A wide range of extractants have been used but few have been extensively investigated as far as effectivity. As well as those mentioned earlier[6,7] the group at NASA have published a series of reports[41–43] and at an

[26] K. W. Hutchison and R. S. Hansen, *J. Bacteriol.* **119**, 70 (1974).
[27] W. W. Forrest, *J. Bacteriol.* **90**, 1013 (1965).
[28] B. Chin and I. A. Bernstein, *J. Bacteriol.* **96**, 330 (1968).
[29] C. Edwards, M. Statham, and D. Lloyd, *J. Gen. Microbiol.* **88**, 141 (1975).
[30] L. Huzyk and D. J. Clark, *J. Bacteriol.* **108**, 74 (1971).
[31] I. Vlodavsky, M. Inbar, and L. Sachs, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1780 (1973).
[32] R. Wahl and L. M. Kozloff, *J. Biol. Chem.* **237**, 1953 (1962).
[33] E. Egberts, P. B. Hackett, and P. Traub, *J. Virol.* **22**, 591 (1977).
[34] Y. Tsuchiya and H. Sugai, *Biochem. Med.* **28**, 256 (1982).
[35] P. McWalter, *J. Appl. Bacteriol.* **56**, 145 (1984).
[36] R. Guerrero, M. Llagostera, A. Villaverde, and J. Barbé, *J. Gen. Microbiol.* **130**, 2247 (1984).
[37] G. E. Thomas, S. Levitskyy, and H. Feinberg, *J. Mol. Cell Cardiol.* **15**, 621 (1983).
[38] J. E. Cairns, S. G. Nutt, and B. K. Afghan, *in* "International Symposium on Analytical Applications of Bioluminescence and Chemiluminescence" (E. Schram and P. Stanley, eds.), p. 303. State Printing and Publishing, Westlake Village, California 91361, 1979.
[39] R. D. Gruenhagen and D. E. Moreland, *Weed Science* **119**, 319 (1971).
[40] T. J. Clegg and J. L. Koevenig, *Bot. Gaz.* **135**, 368 (1974).
[41] E. A. Knust, E. W. Chappelle, and G. L. Picciolo, *in* "Analytical Applications of Bioluminescence and Chemiluminescence," p. 27 (and other references). NASA Document SP-388, 1975.
[42] G. L. Picciolo *et al.*, *in* "Applications of Luminescence Systems to Infectious Disease Methodology." Goddard Space Flight Center, Greenbelt Maryland, Document X-726-76-212, 1976.
[43] E. W. Chappelle, G. L. Picciolo, and J. W. Deming, this series, Vol. 57, p. 65.

early date highlighted the fact that bacterial ATP turns over rather quickly.[44] A number of comparative studies have been made for environmental samples (marine waters, etc.).[45-47]

In general, many bacteria have ATP levels around 1 fg (10^{-15} g) per cell. Yeasts have around 100 times more and many animal cells contain around a picogram ATP (10^{-12} g). Some actual values are given in refs. 1–3.

I will now consider, albeit briefly, the main extractants and give a few references wherein details can be obtained.

1. Boiling buffer, usually Tris–Cl, with EDTA; marine microbial samples,[2] freshwater microbial samples,[48] nematodes,[49] yeast,[50] rumen contents,[12] mycoplasmas,[23] mycobacteria.[22]
2. Various dilute acids including nitric acid (mainly clinical samples,[41-43] sulfuric acid [seawater samples,[2,45,47] clinical samples (ref. 47, p. 189)], perchloric acid (microbial samples,[14,27,51] erythrocytes,[34] tumor,[33] plants[52]), trichloroacetic acid (microbial samples,[6,7] somatic cells[3,7]), and formic acid (microbial samples[44]).
3. Organic compounds including dimethyl sulfoxide (bacteria),[26] ethanol (alga),[53] acetone (yeast),[54] chloroform (mycobacteria),[55,56] and butanol (bacteria).[57]
4. Surfactants including Triton X-100 (somatic cells)[3,6,7] and benzalkonium chloride (yeast).[58]

In addition various mixtures have been described which include some of those extractants mentioned above and which have been used for complex samples such as soil.[10,11] Further a number of commercial extractants of undisclosed content are available but little has been published about their efficiency in comparison with those listed above.[4,56]

In all cases it is important not to overload the extractant with too much sample. Usually a few milligrams dry weight of sample per milliliter of extractant is satisfactory. It is also important to check the sampling/extraction procedure to ascertain that the process itself does not change the ATP level or influence the measured result. An example of poor sampling might be in drawing a blood sample from an animal and allowing the sample to hemolyze, thereby releasing ATP into the plasma. Another might be to allow a microbial sample to become nutrient deficient during sampling.

As mentioned previously there have been few critical studies in this area. One of the first[6] involved the use of 5 microbial species with 10 different extractants. From this study, the workers concluded that only trichloroacetic acid (TCA) was entirely satisfactory for all species. In a follow up,[7] 5 different extractant systems were tested on 7 species of microorganisms and 9 different types of somatic cells. From both studies it was concluded that trichloroacetic acid should be the standard against which other extractants should be tested. In the second of the studies the authors suggest 10, 5, 2.5, and 1.25% TCA (final concentration: equal volumes of TCA and sample) should be employed initially as a standard before searching for better extractants of ATP. In coming to a decision, the total luminescence as well as the lowest degree of quenching of luciferase by the extractant need to be taken into account when choosing operational parameters. It is not possible to cover all types of extractants and their use in a wide range of cells. I will therefore indicate some guidelines to follow when designing extraction protocols.

Somatic Cells

Cells which are separate from one another (e.g., blood cells, certain tissue cultures) do not generally need special treatment prior to extraction and can be mixed directly with the extractant. An exception would be a case in which extracellular (free) ATP was present in amounts that could not be neglected and here cells would need washing or the ATP removed enzymically. However, cells that are formed together in a more or less solid tissue from living organisms (e.g., muscle, kidney, plant leaves) should be frozen to stop the action of ATPases and then homogenized or thinly sliced (<0.2 mm). Freezing is best done in liquid nitrogen and homogenizing with a blender or a simple mortar and pestle. Slicing can be done with a razor blade or on a cryomicrotome.

If small amounts of ATP are expected it is important that equipment be kept scupulously clean and free from microbial contamination. Follow-

[44] W. Klofat, G. Picciolo, E. W. Chappelle, and E. Freese, *J. Biol. Chem.* **244**, 3270 (1969).
[45] O. Holm-Hansen and D. M. Karl, this series, Vol. 57, p. 73.
[46] "ATP Methodology Seminar" (G. A. Borun, ed.). SAI Technology Co., San Diego, California, 1975.
[47] "2nd Bi-Annual ATP Methodology Symposium" (G. A. Borun, ed.). SAI Technology Co., San Diego, California, 1977.
[48] B. R. Taylor and J. C. Roff, *Freshwater Biol.* **14**, 195 (1984).
[49] H. J. Atkinson and A. J. Ballantyne, *Ann. Appl. Biol.* **87**, 167 (1977).
[50] A. Cockayne and F. C. Odds, *J. Gen. Microbiol.* **130**, 465 (1984).
[51] M. Statham and D. Langton, *Process Biochem.* **10**, Oct. 25 (1975).
[52] P. E. Stanley, *in* "Liquid Scintillation Counting" (M. A. Crook and P. Johnson, eds.), Vol. 3, p. 253. Heyden and Son, London, 1974.
[53] J. B. St John, *Anal. Biochem.* **37**, 409 (1970).
[54] L. F. Miller, M. S. Mabee, H. S. Gress, and N. O. Jangaard, *J. Am. Soc. Brew. Chem.* **36**, 59 (1978).
[55] A. M. Dhople and E. E. Storrs, *Int. J. Leprosy* **50**, 83 (1982).
[56] R. P. Prioli, A. Tunna, and I. N. Brown, *Tubercle* **66**, 99 (1985).
[57] E. C. Tifft, Jr., and S. J. Spiegel, *Environ. Sci. Technol.* **10**, 1268 (1976).
[58] M.-R. Siro, H. Romar, and T. Lovgren, *Eur. J. Appl. Microbiol. Biotechnol.* **15**, 258 (1982).

ing homogenization or slicing, an aliquot of the sample can then be allowed to thaw in the presence of the extractant so long as they are well mixed and no clumps of tissue are formed. It is essential that extractant be quickly and intimately associated with all cells. If the cells clump together the extractant may take a long time to penetrate to the inner cells by which time considerable changes in ATP content may have taken place. Alternatively the final result may be too low because little or no ATP has been extracted from those cells. The problem of clumping may be solved in some cases by subjecting the extracting sample to ultrasound.

Alternatively tissue samples may be homogenized at ice water temperature in a glass–glass homogenizer, blender, etc. Another approach would be to homogenize the sample in boiling buffer (e.g., nematodes[49]). For large samples of tissue, 10 g tissue in 100 ml buffer may be homogenized as described in ref. 59 at room temperature or in a cold room.

Microbial Cells

Bacterial cultures can often be added directly to the extractant. For acidic extractants 1 ml culture to 1 ml acid is generally satisfactory and if boiling buffer is used then 1 ml culture into 10 ml boiling buffer should be employed.

Fungal cultures with mycelia and algal cultures with multicellular filaments may require homogenizing in a blender in the presence of the extractant.[60]

Because yeast cells and microbial spores have thicker walls than bacteria they consequently often require more drastic extractants or a longer extraction time.

When yeast and bacteria are both present in the sample (e.g., urine) one method of measuring both is to use a differential filtration procedure.[61] I am not aware of any extraction procedures which permit differentiation of two or more microbial species in the same sample without complex manipulation, e.g., cell separation or growth in selective media.

When microbial numbers are small a concentration step may be required and filtration or centrifuging may be convenient. Both methods tend to lead to a decrease in ATP content because of oxygen and nutrient depletion. The problem can be usually solved by adding a small volume of a suitable culture medium to the filter or pellet and allowing the microbial cells to recover for 5–10 min before extracting them.

[59] C. J. Stannard and P. A. Gibbs, *J. Biolumin. Chemilumin.* **1**, 1 (1986).
[60] N. A. Hendy and P. P. Gray, *Biotechnol. Bioeng.* **21**, 153 (1979).
[61] T. S. Tsai and L. J. Everett, *in* "Analytical Applications of Bioluminescence and Chemiluminescence" (L. J. Kricka, P. E. Stanley, G. H. G. Thorpe, and T. P. Whitehead, eds.), p. 75. Academic Press, London, 1984.

Somatic and Microbial Cells Mixed Together with Nonliving Material

This is the most complex system. Examples in this section include soil,[4] nematodes in soil,[49] meat,[59] milk,[62] rumen contents,[12] fruit juices,[63] and clinical samples.[1,3,42,43]

Suppose you wish to determine microbial ATP in one of the above samples. In most situations the somatic ATP will be present in large excess (perhaps 10^6-fold) and this can pose considerable technical problems, especially if a number of samples are to be processed. Two approaches have been used. In the first, somatic cells are selectively extracted (e.g., using Triton X-100) and the somatic ATP together with any free ATP can then be hydrolyzed with an ATPase.[43] The second method involves physical separation of somatic and microbial cells by differential centrifuging or filtration or addition of resin.[59] In both cases the remaining microbes can then be extracted.

As far as differential extraction is concerned use is generally made of the substantial difference in cell wall/membrane in microbial and somatic cells. Care however must be exercised since so-called somatic extractants (e.g., Triton X-100) have been shown to extract ATP from some bacteria (e.g., *Pseudomonas aeruginosa*.)[7] Microbial extractants will in most cases also extract ATP from somatic cells.

There are two other sources of ATP in many samples. First, free ATP, that is ATP which is in true solution. This will generally be measured and not distinguished from cellular ATP unless it is removed enzymically or by washing. Second, there is the more problematic "bound" ATP which is sequestered on protein and other macromolecules and surfaces in a fairly firm fashion. Bringing the pH of the sample below 4.5 using malic acid has been used to release this ATP so that intereference by it can be removed.[43]

Some Other Points Concerning Extractants

The use of boiling buffer is popular because of simplicity and is quite satisfactory if very high sensitivity is not required. If sensitivity is a requirement then the dilution involved (usually 10-fold) is a disadvantage. Be sure to have the buffer boiling before addition of sample (at least 1 : 5, or better 1 : 10 sample : buffer) so that enzyme denaturation is immediate. If it is not ATPases and other enzymes will change the ATP content even if they are active for say 10 sec.

It is best to avoid as much as possible any postextraction processing as this inevitably leads to losses of ATP during manipulation and to prob-

[62] R. Bossuyt, *Milchwissenschaft* **36**, 257 (1981).
[63] J. G. H. M. Vossen and H. D. K. J. Vanstaen, *Forum Mikrobiol.* **4**, 280 (1981).

lems of contamination because of the ubiquitous nature of ATP (e.g., in sweat, contaminating microbes, etc.) which may be a problem if you are working at high sensitivity.

The use of perchloric acid has led to two reports which indicate other types of problems. Instead of adding the perchloric acid extract directly to the firefly assay system as is usually the case it is possible to reduce the quenching caused by the acid if one removes the acid by precipitating it as the insoluble potassium salt and finishing with a sample at neutral pH. However, one report indicates that some ATP coprecipitates with potassium perchlorate so that final ATP measurements are too low.[64] Another set of workers have reported that on neutralizing perchloric acid extracts from *Bacillus brevis* a phosphatase is reactivated which then proceeds to hydrolyze extracted ATP![65]

If organic solvents are used, some of them may be readily evaporated and this provides an easy way to increase sensitivity should that be necessary.[55]

[64] S. Wiener, R. Wiener, M. Urivetzky, and E. Meilman, *Anal. Biochem.* 59, 489 (1974).
[65] J. A. Davison and G. H. Fynn, *Anal. Biochem.* 58, 632 (1974).

[3] Detection of Bacteriurea by Bioluminescence

By BRUCE A. HANNA

Urinary Tract Infection

Microbial colonization of the urogenital tract, resulting in bacteriurea, is an increasingly common event in modern medical management. While normally sterile, the urinary tract which includes the kidneys, ureters, urinary bladder, and urethra may readily become infected with a wide variety of microbes. Frequently, such infections are preceded by instrumentation and manipulation of the urinary tract, often by insertion of a urinary catheter. As a result, normal flora microbes, particularly skin and enteric bacteria, may gain entry to urinary tract tissues. The severity of such infections may range from those which are asymptomatic except for the presence of bacteriurea, to overt clinical infections of the bladder (cystitis) or kidney (pyelonephritis) which may be accompanied by severe systemic symptoms. Evaluation of the urine to determine the presence and concentration of microorganisms in the urine is an important adjunct in the diagnosis and treatment of urinary tract infections.

Significant Bacteriurea

The definition of what represents clinically significant bacteriurea has been the subject of much discussion. In actuality, the criteria for defining significant bacteriurea is dependent on the patient from whom the sample is derived.[1] The conventional criterion invoked to detect urinary tract infections in asymptomatic patients where there is a low prevalence of disease in the population, is 10^4–10^5 colony-forming units (CFU)/ml in a freshly voided, first morning specimen.[2] In this select patient population counts of $>10^5$ are almost certainly significant, while counts of $<10^4$ have a high probability of representing urethral contamination. In symptomatic patients, on the other hand, where the prevalance of urinary tract infection in the population is high, colony counts of 10^3 or even 10^2 CFU/ml may be considered significant.[3] Frequently in such patients the urine will contain numerous polymorphonuclear leukocytes and other blood cell components as well as bacteria. In addition to bacteria, urinary tract infections may on occasion be caused by fungi, especially *Candida* species, *Chlamydia trachomatis, Ureaplasma urealyticum, Mycoplasma hominis, Mycobacteria* and viruses.

Detection of Bacteriurea

The techniques available to determine the presence and quantity of microorganisms in a urine specimen can be divided into those that are growth dependent and those that are non-growth dependent. Growth-dependent methods, by definition, require dilution, inoculation onto a suitable medium, and an incubation period of 18–24 hr. This will result in an enumeration as well as a prelude to the identification of the microbes present. Non-growth-dependent methods, in contrast, do not require cultivation of the organism, but rather provide a direct enumeration of the bacterial population present. Since the noncultivation methods provide the user with a quantitation but not an identification. they are termed screening tests.

In a typical clinical microbiology laboratory as many as 70% or more of urine specimens may not contain significant populations of bacteria. In such a setting, bacteriurea screening tests are very useful in eliminating these samples from further analysis. Intrinsic to these methods is their ability to rapidly identify such samples on the same day as they are collected from the patient. Conversely, as the majority of urinary tract

[1] R. C. Bartlett and R. C. Galen, *Am. J. Clin. Pathol.* 79, 756 (1983).
[2] R. Plott, *Am. J. Med.* 75(1B), 44 (1983).
[3] W. E. Stamm, G. W. Counts, K. R. Running, S. Fihn, M. Turck, and K. K. Holmes, *Am. J. Med.* 307, 463 (1982).